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EFFECT OF EUTROPHIC WATER ON COLOR AND REPRODUCTION OF COMMON DUCKWEED, *Lemna minor* (Araceae: Lemna)

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Abstract

A type of water pollution with the excess amount of nutrients is defined as eutrophication. People seem to take the phenomenon inconsequential matters due to rarely news hear about the consequences of eutrophication. *Lemna minor* purify the water contaminant and act as indicator. Since there is small extent to which people know about the effect of eutrophication towards the common duckweed, this study has been conducted. The aim of this research to measure the concentration of phosphate that led to eutrophication from different types of water sources and to determine impact of different type of water sources on the reproduction of new fronds and color of *Lemna minor*. The results revealed that fertilizer run-off has the highest reduction of phosphate concentration with 50 ppm (28.74%). While, the highest removal efficiency of phosphate concentration were found with 55.17% for fertilizer run-off in comparison to the removal efficiency of phosphate concentration 42.85% and 0% in tap water and leachate sample respectively. The observed changes in fronds color of all water samples shows that they lost the chlorophyll due to its maturity and salt stress at different types of water samples. Results showed that tap water produced the highest number of new fronds compared to fertilizer run-off and leachate sample. In conclusion, the concentration of phosphate that lead to eutrophication from different type of water sources and the impact of different type of water sources on the reproduction of new fronds and color of *Lemna minor* was obtained.

Keywords: *Lemna minor*, eutrophication, concentration of phosphate, reproduction, color

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Introduction

One of the most difficult issues at the present is environmental degradation, which has gained popularity. Water pollution is one of the worst types of pollution. According to Haseena *et al.* (2017), lower water quality due to material discharge into water bodies is known as water pollution. The water bodies are being polluted by terrestrial run-off, municipal and industrial effluents, inorganic toxicants such as heavy metals. Other than that, the excess use of fertilizers and the aimless practice of pesticides in agriculture give rise to water pollution (Häder and Erzinger, 2018). There are many form of water pollution such as nutrient pollution, chemical pollution and oil pollution (Muralikrishna and Manickam, 2017). This study was focusing on nutrient pollution which can lead to phenomenon that called as eutrophication.

A broad environmental change called eutrophication often diminishes the stabilising impact of plant variety on local production (Hautier *et al.,* 2020). Eutrophication occurs when excess of nutrients exist in water body and can lead to boost vegetative growth (Istvánovics, 2009) and promote the growth of cyanobacteria which is the key symptom of this event (Istvánovics *et al.,* 2022). The two principal nutrients that collect on the surface of lakes, rivers, and base sediments are nitrogen and phosphorus. This activity naturally occurs in lakes and ponds at a stagnant as organic materials accumulate during ecological progression (Aoki, 2012). Yet, it can harm the ecosystem as it accelerates drastically through human activities. Run-off from agricultural areas, detergent waste in washing machine drains, and sewage disposal are a few significant sources of nutrients entering lakes or ponds. (Minnesota Pollution Control Agency, 2008). The aquatic plants such as duckweed can be a bioindicator to trace the eutrophication phenomena as it is dominant on the surface of water bodies (Knight *et al*., 2014). *Lemna minor* (Araceae: Lemna) is a type of duckweed that can give a huge impact and effect towards eutrophication (Zhang *et al*., 2014). Hence, this study was conducted to examine different type of water samples that are contributing to eutrophication by measure the concentration of phosphate. The eutrophication rate become increase as human activities such as run-off fertilizers from agriculture areas, industrial and municipal wastewater discharge contribute the worst effect through runoff of nutrients including phosphorus and nitrogen into aquatic ecosystems (Bashir *et al.*, 2020).

In Malaysia especially at urban area such as Negeri Sembilan, it has been observed that this place contribute to the eutrophication from both point and non-point sources. Point source which are discharged from industries, sewage treatment plant, animal feedlots and oil industries while the non-point sources contains pollutants from runoff from agriculture, pesticides and animal waste (Rasalingam *et al*., 2014). All the sources were discharge from agriculture pond at area of Terachi, Kuala Pilah and leachate from Pajam, Nilai. The excess enrichment of nutrients has become the major problem where phosphorus is the main factor of this phenomenon. The water quality becomes low due to the depletion of dissolved oxygen. Thus, the biological oxygen demand rate will be increased through the respiration process from the uncontrolled algal growth (Hrycik *et al*., 2016). As a consequence, the aquatic animals and plants can die due to depletion of dissolved oxygen over time. There is a lack number of data on eutrophication in Malaysia especially in Negeri Sembilan thus the comparison on the previous data is unable to be obtained.

Lemna minor act as bioindicator to indicate the level concentration of phosphate in the sources that can lead to eutrophication. The objective of this study is to provide information on how much concentration of nutrient give a huge impact on eutrophication. This research can shed light on how *Lemna minor's* morphological traits and reproduction may be impacted by various solutions with various components. The water pollution can be reduced as the water quality being improved when there is presence of biological control agent or water purifier. Other than that, the data also can be used as a reference source by the government to treat surface and underground water pollution as well as soil by carrying out bioremediation processess and others. Additionally, this study can contribute to data expansion while simultaneously publishing data for others to use as a guide. Eutrophication, which has severe effects on aquatic life like fish, which are essential for economic value and as a source of protein, will create awareness among the community.

Materials and Methods

Water Sample Collection

This research was carried out at UiTM Negeri Sembilan Kampus Kuala Pilah (UiTMCNS). About three different water samples including tap water (control) was acquired from UiTMCNS, fertilizer waste disposal from agriculture area was collected at Terachi Paddy field, Kuala Pilah and landfill leachate samples was collected from Landfill Pajam, Nilai. About 80 litres of each water samples were collected.

Preparation of Experiment and Mother Plant of *Lemna minor*

About six containers (44 cm x 31.8 cm x 28 cm) were prepared for each water samples from different sources. The other different container (10 cm x 10 cm x 10 cm) was filled with distilled water (1 litres). At first, 90 individuals mother plant of *Lemna minor* were placed into the container (10 cm x 10 cm x 10 cm) for two days to adapt with zero nutrients (Figure 1). Later, the mother plants were transferred into the other container (44 cm x 31.8 cm x 28 cm). The preparation was conducted outside the laboratory.

Figure 1. Preparation for mother plant of *Lemna minor*

Preparation of First Replication

About 40 litres of tap water was filled into the container (44 cm x 31.8 cm x 28 cm). After that, phosphate concentration of tap water was measured using High Range Phosphate Colorimeter (HI713 Checker® HC) and was recorded. The container was divided into 15 compartments (Figure 2). Only one individual of mother plant was placed in each compartment for the observations on new frond germinations and color changes. Similar processes were conducted for the other water sample types.

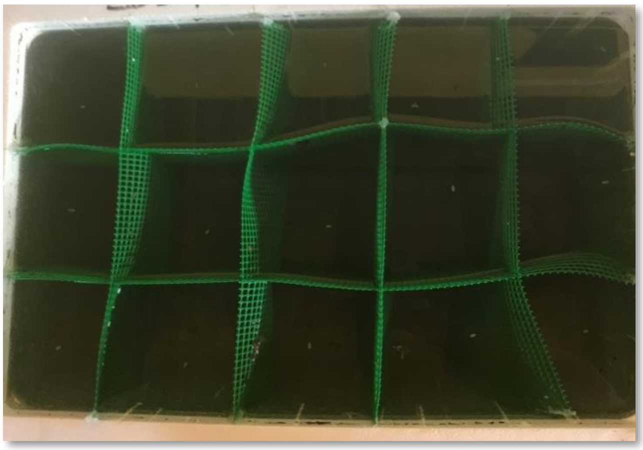

Figure 2. Preparation of first replication

Phosphate Concentration Measurement

The phosphate concentration of water was measured on day 10, day 20 and day 30. About 10 millimeters (ml) of water samples were collected and was tested using High Range Phosphate Colorimeter (HI713 Checker® HC) (Figure 3).

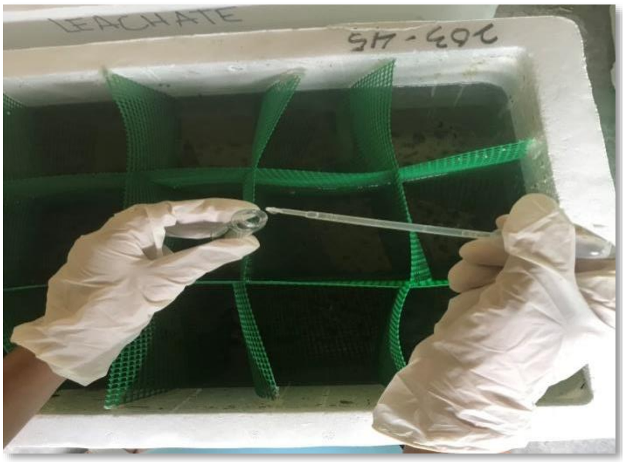

Figure 3. Measurement of phosphate concentration

Observation on New Frond Germinations of *Lemna minor*

The new fronds germination of *L. minor* were observed and recorded every day until day 30. The observations were conducted at 9 am and 7 pm. The collections data were recorded and were analyses using Analysis of Variance (ANOVA).

Observation on Color Changes of *Lemna minor*

Each individual of *L. minor* was picked randomly to observe the frond color changes using dinolite microscope at day 1, day 10, day 20 and day 30. Then, the collections data were recorded.

The Process of Experiment

Similar processes as above were conducted for the other two water samples including leachate sample and fertilizer waste disposal, and each type of sample were replicated twice.

Data Analysis

The data was recorded in the Microsoft Excel 2013 and Microsoft Word 2013. While Minitab 17 was used for analyzing one-way ANOVA. The formula used is:

$$\text{Removal efficiency (\%)} = \frac{(c0-c) \times 100\%}{c0}$$

Where:
c0: initial pollutant concentration (ppm)
c: real-time pollutant concentration (ppm)

Result and Discussion

Phosphate Concentration Measurement

Figure 4 demonstrates the phosphate concentrations (ppm) between tap water (T), fertilizer run-off (F) and leachate sample (L). The tap water has declined slightly in the phosphate concentration. In the first day, the phosphate concentration is 42 ppm (100%) where there was no reduction of phosphate recorded. The next 10 days which is day 10, the value is 40 ppm (95.23%) with 4.76% phosphate reduction. Meanwhile at day 20 the phosphate concentration is 38 ppm (90.48%) and involves the phosphate reduction with 4.76%. Then it suddenly decreased in the final days of the experiment which is 24 ppm (57.14%) with 33.33% phosphate concentration reduction. It shows that the highest reduction of phosphate concentration recorded in tap water is in the final day with 33.33%.While, the fertilizer run-off from the paddy field also has dropped dramatically from 174 ppm (100%) to 124 ppm (71.26%) which involves reduction of phosphate concentration with 28.74%. Then, 105 ppm

(60.34%) phosphate concentration was recorded at day 20 with 10.92% phosphate concentration reduction. After that, it fell gradually to 78 ppm (44.83%) during the last day which involves reduction of phosphate concentration as much as 15.52%. During day 10 of the experiment, it was recorded that the highest level of phosphate concentration reduces as much as 28.74%. Lastly, the leachate concentration has remained constant at 200 ppm (100%) with no reduction of phosphate concentration. Overall, the phosphate concentration among three different types of water samples shows the highest reduction on the fertilizer run-off is about 50 ppm (28.74%) within day 1 to day 10 of the experiment. Meanwhile, the removal efficiency of phosphate concentration was calculated using the formula that has been stated. The removal efficiency for the tap water is 42.85% while for fertilizer run-off from the paddy field is 55.17%. However, the leachate removal efficiency is 0% since there are no differences between initial concentration and current concentration. In addition, it can be observed that fertilizer run-off has the highest removal efficiency of phosphate concentration which is 55.17%.

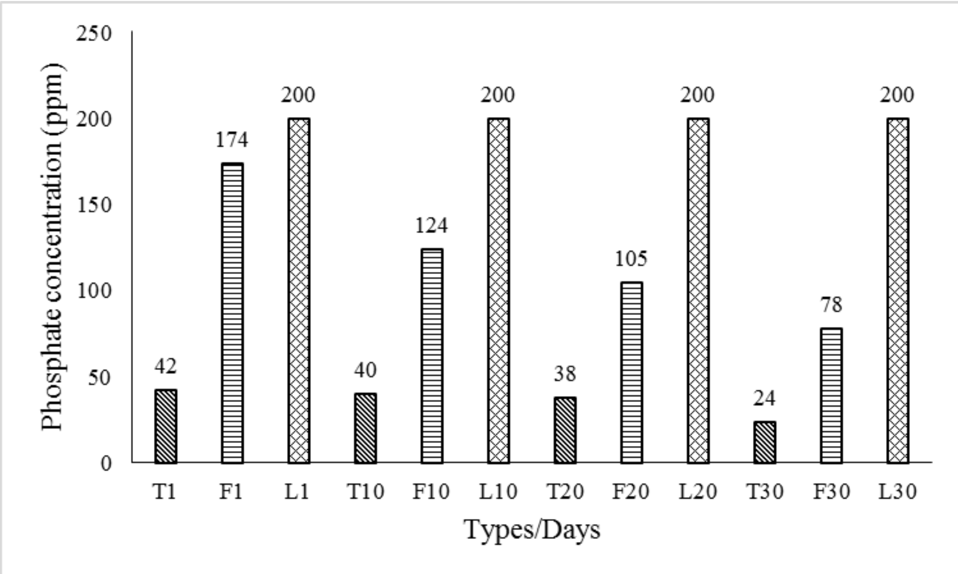

Figure 4. Phosphate concentrations (ppm) between three different types of water samples at day 1, day 10, day 20 and day 30

The tap water has the constant reduction rate from day 1 to day 20 since they have the same number of individual. They also could absorb the same amount of phosphate concentration due to the same growth and development between day 1 to day 20. While at day 30 of the experiment, the reduction of phosphate concentration is gradually increased due to the maturity of the fronds. The upcoming matured fronds need to absorb large amount of phosphate in order to grow healthy and produce more number of frond. The highest level of phosphate concentration reduce is 28.74% on day 10 of the experiment. The phosphate concentration in fertilizer run-off has the highest number of reduction because of *L. minor* are able to absorb more phosphate within day 1 to day 10. The high phosphate concentration in the fertilizer run-off forced the *L. minor* to absorb the nutrient. This result was supported by Chen *et al*. (2018) that stated the duckweed removed the overabundance nutrients in the water bodies and the excess amount of phosphorus that is known as main pollutants in the water bodies that can lead to eutrophication. Besides that, the phosphate concentration for leachate sample remains constant at 200 ppm (100%) and removal efficiency is 0% since there is no change in 30 days. The phosphate concentration was recorded the highest concentration but the phosphorus concentration is not reduced and absorbed by *L. minor*. Phosphorus in landfill leachate cannot be absorb and remove by *L. minor* because it best in removing heavy metals such as copper, zinc and nitrogen. Our result is supported by Daud *et.al*. (2018) where the *L. minor* is the best metal removal as it could removes metal from leachate sample more than 70% to 90%. In addition, the duckweed is very effective in absorbing organic matters, suspended solid and soluble salts from leachate sample.

According to Ström (2010) phosphate levels were lower than the available measuring range (<0.5 mg/L). This shows that no nutrient removal could be recorded for phosphorus. In other words, phosphorus was safe to be absorbed by plants and algae since phosphorus are usually known to be the nutrients necessary for all plant growth. Hence, this bioavailability should not be a problem as phosphorus content in the landfill leachate become harmless and can be uptake by plants. But, the results showed that the phosphate concentration is more than 0.5 ppm which means it has the ability to absorb the phosphate concentration. Somehow, the amount of phosphorus inside the leachate could be low compared to the other component such as zinc, ammnonia and nitrate (Youcai, 2018).

In contrast, Iqbal and Baig (2017) were reported that leachate samples have high concentrations of ammonia (3032 mg/dm^3), nitrate (22 mg/dm^3), nitrite (120 mg/ dm^3) and phosphate (3000 mg/dm3). Besides that, there was recorded the highest phosphate removal is 200 mg/m^2/day in concentrated leachate. The result of the experiments also shows that the phosphate cannot be removed or absorbed by *L. minor* although there is highest phosphate concentration. The obtained result is assumed to be influenced by temperature and light intensity of the current condition.

Observation on Color Changes of *Lemna minor*

A. Tap Water

The color changes of *L. minor* were observed at day 1, day 10, day 20 and day 30. Based on Figure 5, it shows that the fronds in the tap water start to change their color at day 10. The green color of the frond is start to fade away if compare to day 1 of the frond which shows the fresh green color. However, there were also some of the individuals at day 10 that still remain in green color of the whole area of frond. While at day 20, it can be observed that there were more than half of the individuals of *L. minor* that have lost the green color. The frond's color turns to brownish and yellowish. Nevertheless, there are few of the fronds that were still green without changing the color to yellow or colorless. On day 30, the majority of the fronds had completely lost their colour while a few remained unchanged.

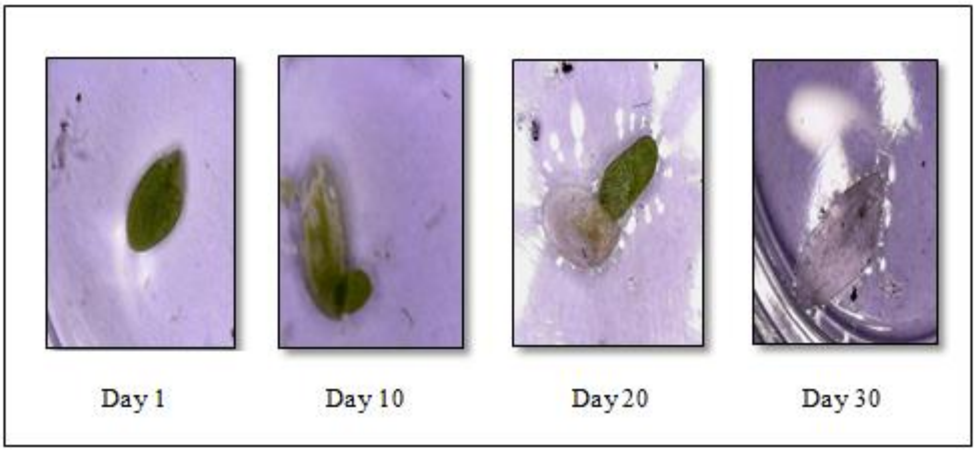

Figure 5. The color changes of *L. minor* in 30 days in tap water

The color of the frond is green due to the content inside the frond. The fronds contain a pigment known as chlorophyll (Ohmiya *et al.,* 2014). There are few individuals that still producing higher amount of chlorophyll which gives the whole frond green in the color even though the other frond already colorless. The frond has completely lost their chlorophyll when the color turns to colorless or other than green color. The presence of chlorophyll makes their color remain green but as mentioned by Paiha (2021), when the frond has reached its maturity as they have produced maximum number of daughter and size, it will demonstrating senescence and eventually die. Some of the frond remains green because the limit of the reproduction does not reached yet. Besides that, if the study is prolonged for few more days, the result will shows the same result as day 30 where all the fronds will turn the color to colorless.

B. Fertilizer Run-off from Paddy Field

Based on Figure 6, all individuals of *L. minor* were green in color from day 1 until day 10. No colors changes occur because the fronds were still consist of chlorophyll. Next, the frond at day 20 was started to reduce the green color where the chlorophyll was begin to break down. Nevertheless, there were also other frond which remain the green color but in small quantity. Throughout 30 days experiment, half part of the fronds has completely changed the color to pale and colorless. Based on the obtained result, both tap water and fertilizer run-off from paddy field shows similar effect on *L. minor*'s coloration. The fronds colors were changed because the chlorophyll inside the fronds was degraded due to lack of light and nutrient deficiency. The chlorophyll gives the leaves or frond the green color. But, based on previous study the fronds color changed because of hunger signs of the frond. Thus, the growth of the duckweed was distracted by the nutrient deficiency as the nutrient is gradually consumed by the duckweed (Chen *et al*., 2018). However, Liu *et al*. (2017) were reported that salt stress in the water samples inhibit the synthesis of chlorophyll that lead to the colorless fronds.

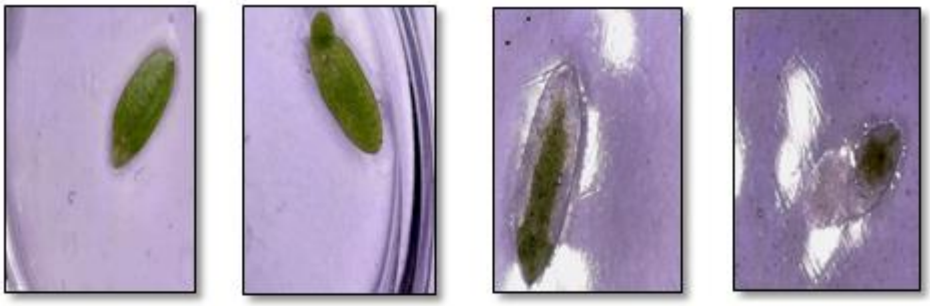

Figure 6. The color changes in *L. minor* in 30 days in fertilizer run-off

C. Leachate

Based on the observations, similar results as fertilizer were shown at day 1 and day 10 on frond's color of *L. minor* in leachate sample. Later, a change in the fronds was observed after 20 days of the experiment where the entire frond has turned their color to brownish. This condition happened to all individuals of *L. minor* in the leachate sample during day 20. Based on the results showed in Figure 7, the frond color was completely changed to colorless. This condition was applied to all the fronds at day 30 of the experiment. The fronds of *L. minor* turned their color from healthy green to pale brown and colorless because of the degradation of chlorophyll in the leachate sample. Besides that, it is possible if the color changes occur due to the amount of pollutant consume by the fronds. The results were supported by Chen *et al*. (2018) the duckweed initially has dark green color and short root. Due to the uptake of pollutant, the color was finally turned to pale and the root of the fronds became long in order to get the nutrient. In comparison, the tap water shows the early reducing number of chlorophyll at day 10 of the experiment. This condition happened maybe because of the maturity of the *L. minor* that makes the chlorophyll break down and changed the color of the frond. Unfortunately, previous study has revealed that the water with low level of nutrient would undergo maximum nutrient starvation (Tao *et al*., 2017). While the fertilizer run-off from paddy field and leachate samples both show the same result which at day 10 of the experiment, they were still remained the whole area of the frond in green color.

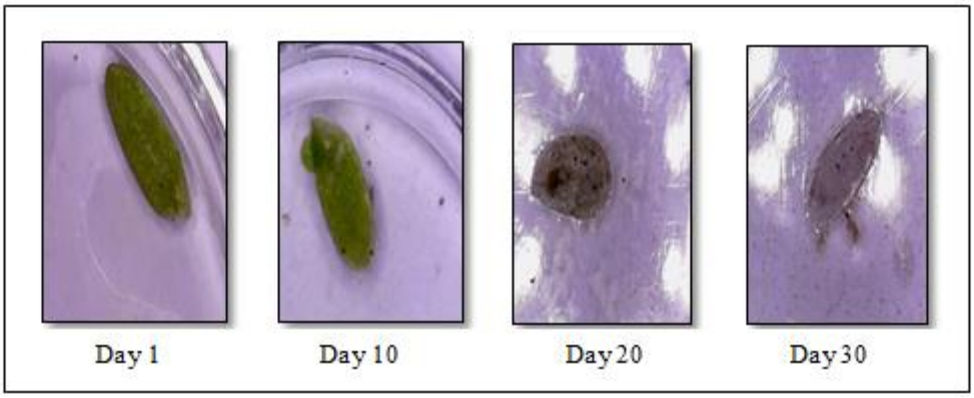

Figure 7. The color changes in *L. minor* in 30 days in leachate sample

From the observation, the color changes of the fronds started from day 20 in both fertilizer and leachate were due to the stress faced by the fronds. For instance, the leachate sample maybe contains the toxic substance which leads to the changes of the fronds color. The fronds consumed the nutrient and the pollutant inside the water samples which lead to alteration of color. This result is same as previous study that stated the leachate from landfill has high number of toxicity with values of EC50;96h in range of from 1.3% to 2.7% leachate (v/v) and cause the frond having nutrient deficiency such as necrosis and chlorosis (Sallenave and Fomin, 1997). Other than that, all the fronds in the different water samples were faced the abiotic stress. The level of nutrient needed is increase since they have absorbed the nutrient inside the water samples.

Observation on New Frond Germinations of *Lemna minor*

A. Tap Water

According to Figure 8, it shows that *L. minor* in tap water undergoes full reproduction cycle where the first frond as mother plant (first frond) produces new daughter asexually by budding process. In this type of water, productions of new daughter frond occur until fourth frond. The ability of mother plant to produce high number of fronds until it reached the maximum reproduction in tap water is due to the excess nutrient absorb by the individuals.

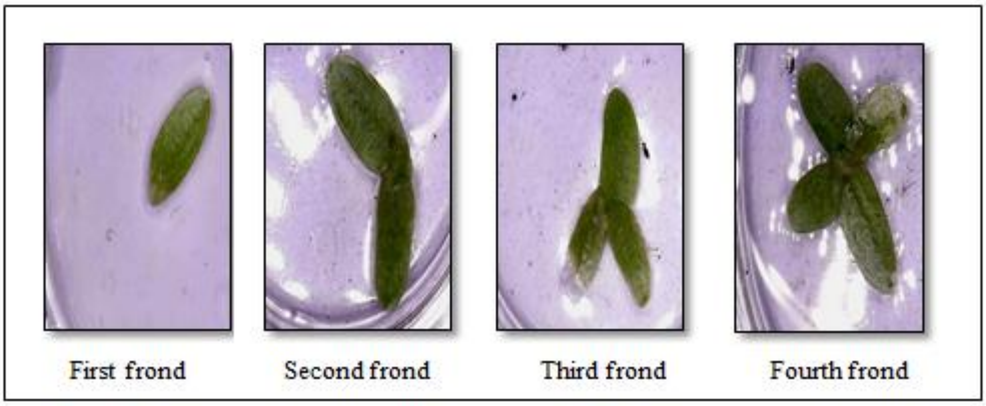

Figure 8. Germination stages of *L. minor* in tap water

Based on Table 1, it demonstrates that total new fronds germinate at day 1, day 10, day 20 and day 30 for both morning and night. The highest germination rate per day shows at day 10 with 13 individuals followed by day 30 (7 individuals), day 20 (3 individuals) and day 1 (0 individual). The overall total number of new frond germinates were 23 individuals. Each of the individual might absorb and get enough nutrient uptakes from tap water. From the data analysis from ANOVA, there is no significant difference in new frond germinations rate of *L. minor* in tap water; morning versus evening $p = 0.825$ ($p > 0.05$).

Table 1. New frond germination in tap water

	No. of frond		Total	
Day	Morning	Evening	Per day	Overall
1	0	0	0	0
10	5	8	13	13
20	1	2	3	16
30	5	2	7	23
Total	11	12		

The germination stages of the *L. minor* in tap water reached the maximum number of new fronds since it getting sufficient supply of nutrient. It shows that the reproduction of the *L. minor* can occur although in tap water because it depends on ability of the species to absorb the concentration of nutrients. The reproduction of *L. minor* in tap water is abundant because it has exceeded the level of optimum phosphorus content which is 1 ppm. Pierre Elliot Trudeau High School (2013) was reported the similar results observed from this experiment which shows the healthy fronds but not with accelerated growth. This result also was supported also by Spring (2013) with the number of duckweed individuals increased from 10 individuals to 14 individuals, 16 individuals, 15 individuals, 17 individuals, and back to 15 individuals in tap water because it supplied with nutrients.

B. Fertilizer Run-off from Paddy Field

Based on Figure 9, it shows that *L. minor* in fertilizer run-off undergoes reproduction cycle where the mother plant produce new daughter by budding process. Most of the individual samples of *L. minor* were produced new frond until second fronds only. But, some of them were able to producing the third fronds. In fertilizer run-off, there was no production of fourth fronds.

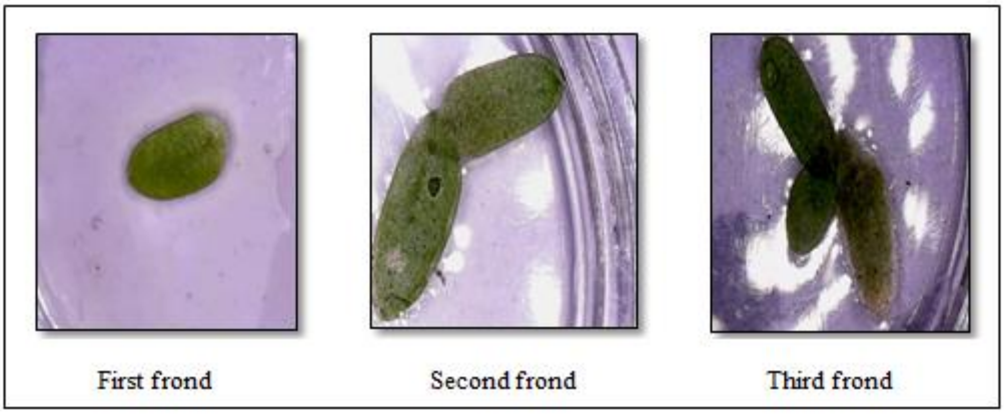

Figure 9. Germination stages of *L. minor* in fertilizer run-off

Based on Table 2, it shows the total new frond germinate at day 1, day 10, day 20 and day 30 in the morning and evening. Based on observations, the highest number of new frond germinates per day recorded at day 10 (14 fronds). While at day 20 (1 frond) and day 30 (2 fronds), the mother plant produce very small number of new frond respectively. The total number of new frond germinates were 17 individuals which give second highest number after tap water. Eventhough each individual of *L. minor* in fertilizer run-off absorb more concentrations of phosphate, but they were unable to reproduce higher new frond number compared to tap water. Other than that, there is no significant differences of new germinations of *L. minor*; morning versus evening with $p = 0.463$ ($p > 0.05$).

Table 2. New frond germination in fertilizer run-off

	No. of frond		Total	
Day	Morning	Evening	Per day	Overall
1	0	0	0	0
10	7	7	14	14
20	1	0	1	15
30	1	1	2	17
Total	9	8		

The germination and the new number of frond produced in the fertilizer run-off were due to the adequate nutrients. The agriculture area fulfills the amount of optimum nutrients required by the duckweed (Chakrabarti *et al*., 2018). However, the result shows the total number of new fronds is quite low. Supposedly, fertilizer run-off can cause the *L. minor* to growth in large amount. According to research done by Pierre Elliot Trudeau High School (2013) the fertilizer can cause disturbance on the growth of the other plant because it can be polluted if there are excess amount of the nutrient. Fertilizer also contain secondary nutrient including calcium and magnesium which means can give the higher rate of *L. minor* reproduction. Hassan and Chakrabarti (2009) was reported that the distinction in climatic conditions and nutritional status of the water body were affected the reproduction of the duckweed.

C. Leachate

According to Figure 10, it shows that *L. minor* in leachate sample undergoes reproduction cycle where the first frond as mother produce new daughter by budding processes same as tap water and fertilizer run-off. However, there were only four number of new germination in leachate during day 10. In addition, there was no production of new daughter frond for day 20 until day 30. The reproduction cycle of *L. minor* in leachate sample ends at frond number two or after producing new first daughter. Assuming *L. minor* cannot germinate or produce new budding because of the pollutant content inside the leachate sample is high and lead to damage the whole structure of *L. minor*.

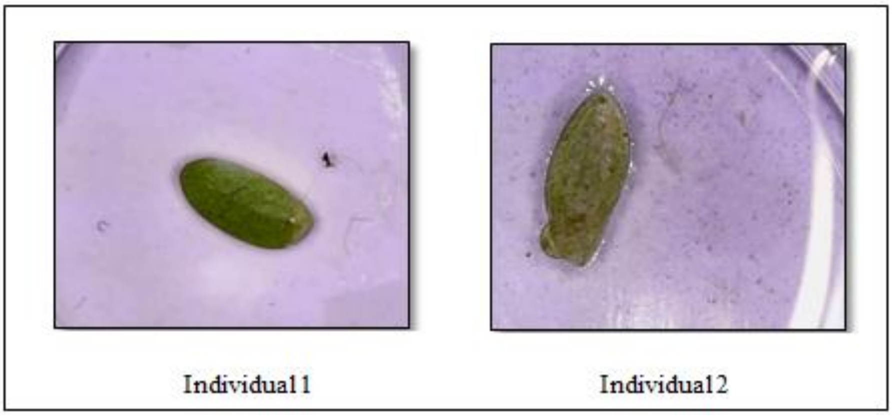

Figure 10. Germination stages of *L. minor* in leachate sample

Based on Table 3, it illustrates that total number of new frond germinate at day 1, day 10, day 20 and day 30 for both morning and night. At day 10 of the germination, it can be seen that it gives only four numbers in total. While at day 20 and day 30 the individual produce none of new frond respectively. The overall total number of new frond germinates were four individuals who placed the lowest number of germination of new fronds compared to tap water and fertilizer run-off. From the analysis of data, there is significant different of new frond germinations of *L. minor*; morning versus evening with $p = 0.045$ ($p < 0.05$).

Table 3. New frond germination in leachate sample

	No. of frond		Total	
Day	Morning	Evening	Per day	Overall
1	0	0	0	0
10	4	0	4	4
20	0	0	0	4
30	0	0	0	4
Total	4	0		

The germination stages and total number of new frond in leachate sample give the very small amount of germinations because of the component in the leachate itself. The leachate is concentrated where it makes the duckweed reproduction retarded. This result was supported by Iqbal *et al*. (2019) where the synthetic or diluted leachate is better in growth and reproduction of *L. minor* than in the concentrated leachate. Besides that, the removal of nutrients such as phosphorus and nitrogen by the other component and factor in the landfill leachate leads to the stunted growth of *L. minor*. Meanwhile, Liu *et al*. (2017) was reported the growth of the *L. minor* influenced by salt stress because it can cause injuries to the duckweed in the long-term cultivation with higher salt stress.

Conclusion

As conclusion, the concentration of phosphate that causes the eutrophication from tap water, fertilizer run-off and landfill leachate were above the recommended maximum contaminant level; 1.0mg/L (1 ppm). The highest reduction of phosphate concentration among three different types of water samples was the fertilizer run-off with about 50 ppm (28.74%). The color changes of *L. minor* towards different types of water samples were affected by the maturity and the content inside the water samples. Other than that, *L. minor* can reproduce and grow in the adequate supply of nutrients but not inside the concentrated leachate. The germination of *L. minor* does not affected by the time since there is no significant difference between the times of the germination except for leachate sample. This study is recommended to be continued by determine the relationship between abiotic factor and the growth of *L. minor*. Other than that, it suggested to repeat this study in order to ensure the similar results.

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Author Contribution

Nur Syafiqah carried out the sampling, result and participated in drafting and writing the manuscript. Nur Hasyimah performed the statistical analysis and helped to draft the manuscript. All authors contributed equally to this work.

Conflict of Interest

Author declares no conflict of interest.

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